In 1890 Mr. S. R. Boyce, my assistant, made a distillation of a large quantity of the ground root. He obtained by this process an oil of a yellowish color, which soon blackened. This oil has a very acrid taste and pungent odor, evidently containing the medical properties of the root in concentrated form.

ROOT TUBERCLES AND THEIR PRODUCTION BY INOCULATION.

BY D. H. OTIS, MANHATTAN.

Read before the Academy October 28, 1897.

HISTORY AND LITERATURE.

GENERAL STATEMENT.—By examining the roots of such plants as clover, alfalfa, beans, and peas, one will usually find, scattered over their exterior surface, tubercles of various sizes and shapes. These tubercles are, with very few exceptions, peculiar to a certain order of plants known as Leguminosæ, and, as far as agricultural plants are concerned, only to the suborder Papilionaceæ. These tubercles are the outgrowths of the plants themselves, and are produced by the action of certain micro-organisms working within the tissues of the root. Formerly, these tubercles were considered abnormal appendages and as injurious to the plants; but later observations revealed the fact that, where these tubercles were wanting, the plants did not make the growth that was made by plants where the tubercles were present. Later examination has brought out the fact that these tubercles are the homes of minute microscopic bacteria, *Bacillus radicicola* Beyer. The bacteria have the remarkable property of taking the free nitrogen of the atmosphere and transforming it into available compounds for plant food. So it is a case of symbiosis, the plant furnishing food and shelter for the bacteria, and the bacteria in turn furnishing the plant with nitrogen. This is what makes the leguminous plants so valuable as soil enrichers, and especially prized for green manuring.

EARLY OPINIONS CONCERNING THE TUBERCLES.—It is just about a century ago that root tubercles became the subject of agricultural inquiry and experimentation. The early ideas were very crude, some supposing the tubercles to be fungi, others lenticels, root branches, swellings caused by insects, and some used them as a part of the description of plants. Even those who took them to be peculiar to the order Leguminosæ entertained widely different views as to their functions. Some thought they were swollen lateral roots used in the absorption of food, or, still better, a storehouse for reserved food material. Others maintained that they were dwarfed roots, while still others classed them as imperfect buds, capable of developing into new plants. About fifty-five years ago Boussingault carried on a series of experiments with a large number of plants, from which he concluded that not even the leguminous plants had the power to obtain free nitrogen from the air. Similar experiments at Rothamsted confirmed Boussingault's conclusions. It should be noted, however, that these experiments were conducted under the conditions of sterilization and enclosure which eliminated the micro-organisms from the soil. Thus it will be seen that the earliest conclusions were very incomplete, and in many cases were the result of mere superficial observation.

Investigation of the structural and etiological phase of the subject was begun in 1816. It was started by Woronin, and he was followed by Eriksson, DeVries, Schindler, Cornu, Mattei, Kny, Prillieux, and, in 1879, by B. Frank. It was about this time that M. Berthelot called in question the accuracy of the conclusion that plants do not assimilate free nitrogen. This stimulated further investigations, the results of which tended to strengthen confidence in the view that these tubercles were the result of the irritation, or stimulation, of some soil organism, but as to the character of this organism there were many diverse opinions. In 1885 Brunhorst came forward with a paper in which he maintained that root tubercles were not caused by organisms but were normal structures. This view received the indorsement of others, and, for a time, shook the confidence in the theory that micro-organisms were the cause of root tubercles. Even Frank forsook his former conclusions. So at the close of what Atkinson calls the second, or middle, period of investigation (about 1886) the etiology of the whole subject still "hangs in the balance."

Recent Investigations.—In 1887 Marshall Ward published the results of a very careful series of experiments in which he proves that root tubercles are caused by some kind of a soil organism, and this view is supported and confirmed by such investigators as Hellriegel, Wilfarth, Lawes, and Gilbert. Some authors give Hellriegel the credit of being the first to discover the true function of root tubercles.

Doctor Salfeld was the first to experiment with this discovery under field conditions, and found that it was possible to increase the number of tubercles on a leguminous plant by inoculation. In 1888 appeared a valuable contribution by Beyerink, in which he names the bacteria causing these tubercles "*Bacillus radicicola*." In the same year appears an article by Vuillemin, in which he agrees with those authors who call the organism a symbiont, but disagrees with others as to its nature. A. Prazmowski, in 1888, claimed that tubercles were the result of a parasitic fungus, but in a year or two later maintained that they were caused by bacteria. This later view was supported by others, as Delphino, Mattei, Laurent, and Frank, the last in 1890 partially returning to his former views.

One of the first records of an American author in connection with this subject is that of Schneider, who, in 1892, published an article on the bacterioids of several species of leguminous plants. In 1891, F. Nobbe, E. Schmid and L. Hiltner investigated the physiological meaning of root tubercles on non-leguminous plants. Nobbe and Hiltner are also the originators of what is known as "pure cultures." They have isolated the bacteria for seventeen different leguminous plants and are now able to grow these artificially. This discovery was first announced before a German agricultural society February 19, 1896. They now prepare these bacteria on a commercial scale and sell them in bottles under the name of "Nitragin." Geo. W. Atkinson of Cornell University, formerly of Alabama, has published in the Botanical Gazette for 1893 (Vol. 18) a history of the subject, together with some original work he carried on while in Alabama. He takes up the biological phase of the subject and gives some plates illustrating the manner in which the bacteria infect the root. Atwater, Woods, and Kedzie have also done some work along the same line.

From what has been published on the subject it is clear that all the problems connected with the assimilation of free nitrogen, through the intervention of root tubercles, have by no means been solved. Even the best authorities seem to disagree on some of the most vital points. However, it is pretty well settled that the tubercles are the result of a micro-organism; but it has been proven that the organism producing tubercles on the pea or bean will not produce tubercles on clover and alfalfa, and *vice versa*. Whether these organisms are different species for different plants, or a modification of the same species, is yet a disputed question. Again, as the organisms attack the root, it is supposed that they exist in the soil, and the question would naturally arise as to whether they could be transported and spread with the soil; and, if so, whether that is the only way, or whether the seed from plants with tubercles will produce tubercles when grown in soil devoid of the organism adapted to that particular plant. To test some of these questions, and others connected with them, experiments were carried on with the soy-bean, *Glycine hispida* Maxim.

EXPERIMENTS IN THE FIELD.

METHODS OF INOCULATION.—Since 1890 soy-beans have been grown at the Kansas Experiment Station, but frequent and numerous examinations of the roots failed to reveal the presence of any nodules or tubercles. Knowing that the Hatch Experiment Station, at Amherst, Mass., had been successful in producing tubercles on the soy-bean, it was proposed that an attempt be made to inoculate the Kansas beans with Massachusetts soil. Two quarts of the soil in which beans had been grown the previous year was ordered by express for immediate use, and a half bushel by freight for additional experiments in the greenhouse. In both cases the soil arrived in a dry, pulverized condition, not unlike the dust in our roads during a dry season. The field experiment was situated on a sandy loam soil with a western exposure, and consisted of two series of three plats each. Series I was planted with yellow soy-beans, in which the plats were treated as follows: Plat A was inoculated with soil, plat B with extract, and plat C was not treated. Series II was a repetition of series I with the exception that the medium green bean, a variety grown at the Hatch Experiment Station, was used instead of the yellow soy. The object was to note whether there was any difference in the production of tubercles between a variety whose seed was obtained from plants grown in Massachusetts soil and seed obtained from plants grown in Kansas soil. Both series were seeded May 29, 1896. Each plat contained three rows, two and one-half feet apart, and each row contained eight hills twenty inches apart. Between the plats was placed a guard row in which the beans were not treated and were planted in drills from two to three inches apart. The arrangement of the series and the plats is shown in the plan on the following page.

On plats A and D about 21^{cc} (25 grams) of the pulverized Massachusetts soil was placed in the bottom of each hill and the beans placed on top of this. Plats B and E were treated with an extract of the Massachusetts soil. This extract was obtained by mixing a quantity of soil with about seven times its bulk of water, stirring thoroughly, and allowing to settle, after which the water was poured off and used for the inoculation. The aim was to use about the same quantity of soil in obtaining the extract as was used on the same number of plants where the soil was applied direct. Rows 1 and 4 of plats B and E respectively were inoculated at the time of planting, *i. e.*, about 170^{cc} (168 grams) of the extract was poured in the bottom of each hill just previous to planting the beans. Rows 2 and 5, 3 and 6, were inoculated June 13, seven days after the plants were up, and rows 3 and 6 were again inoculated on July 2 and on July 17, or twenty-six and forty-one days respectively after the plants appeared above ground. The extract reached the roots through a round hole made with a pointed stick. Plats C and F were planted in the same manner as the others except the inoculation. The purpose of these plats was to serve as a check on the others and at the same time as a means of comparison with the inoculated plats as regards growth and general appearance.

CULTURE AND GROWTH.—The season was favorable to the growth of the beans. A heavy rain fell the next day after planting, and subsequent rains fell at intervals sufficiently close together to supply the plants with the necessary moisture. The beans were up June 6 and on June 13 all the plats received a

SERIES I: A, inoculated with soil; B, inoculated with extract (1) at time of planting, (2) once after planting, (3) three times after planting; C, not treated.

				1	2	3				
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o	Guard row	o	o	o	Guard row	o	o	o
	—A—				—B—				—C—	
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o

SERIES II: D, inoculated with soil; E, inoculated with extract (4) at time of planting, (5) once after planting, (6) three times after planting; F, not treated.

				4	5	6				
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o	Guard row	o	o	o	Guard row	o	o	o
	—D—				—E—				—F—	
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o
o	o	o		o	o	o		o	o	o

thorough hoeing. On the latter date some of the extra plants were pulled up and there were found several well defined nodules on the roots of those inoculated with soil, but none were found on any of the others at this date. On June 22 it was noted that the beans inoculated with soil appeared to have a little larger growth. The difference was not very striking, however. On July 14 the yellow soys were in full bloom, but the medium green, being a little later variety, did not appear in full bloom until July 20. Measurements were taken for the average height of the plants on August 20, with the following results:

TABLE I.

Plat.	Row 1.		Row 2.		Row 3.		Av. for plat.	
	Ft.	In.	Ft.	In.	Ft.	In.	Ft.	In.
Yellow Soy:								
A	.....	.....	.....	.....	.....	.....	2	8.5
B	2	9.5	2	7	2	4.5	2	7.0
C	.....	.....	.....	.....	.....	.....	2	5.5
	Row 4.		Row 5.		Row 6.		Av. for plat.	
Medium Green:								
D	.....	.....	.....	.....	.....	.....	2	4.0
E	2	2	2	2	2	.5	2	1.0
F	.....	.....	.....	.....	.....	.....	2	0.0

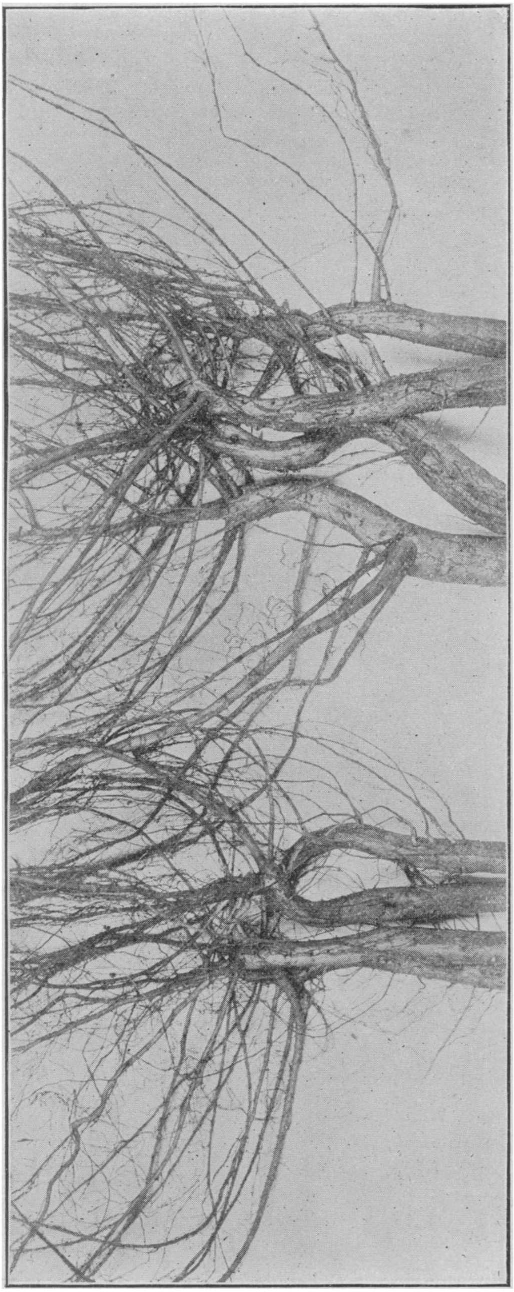
From the above table, it will be noticed that the yellow soy attained a greater height at this date than the medium green. This is due, however, to the difference in the variety, the latter being a late-maturing and a somewhat more bushy plant than the yellow soy. It will also be noticed that, in case of plats B and E, rows 1 and 4, inoculated at time of planting, attained, on the whole, a little greater height than rows 2 and 5, and 3 and 6, inoculated subsequently to the time of planting. This would indicate that the best time to inoculate is at the time of planting. Furthermore, the last column of the table shows, in this case at least, that the plants inoculated with soil averaged a little greater height than the others. However, the differences above noted are not great, and, with the exception of the difference due to variety, would not be noticed by the ordinary observer without the application of a measuring-rod.

Appearance of the Roots.—On August 27 two hills each of the treated plats and one of the untreated were dug up, together with about a two-foot cube of the soil surrounding each hill. These were placed in large tubs of water and after a thorough soaking the roots were carefully washed out and examined for tubercles. The latter were found in great number and of a large size on the inoculated plants; but not a single tubercle could be found on the plants not treated, from either the yellow soy or the medium green, nor were there any signs of tubercles on the plants in the guard rows between the inoculated plats. The tubercles on the plants inoculated with soil were fairly uniform and situated mainly on the upper portion of the roots, not far from where the soil was placed at the time of planting. In case of the plants inoculated with extract there was a marked difference between the varieties; the tubercles on the yellow soy were very numerous and well developed, while those on the medium green were scanty and rather inferior. All the inoculated plants showed a greater diameter of the lower portion of the stem than the plants not treated. Pictures were taken of the different treatments and are here given.

—

Transactions Kansas Academy of Science, Vol. XVI.

PLATE VI.

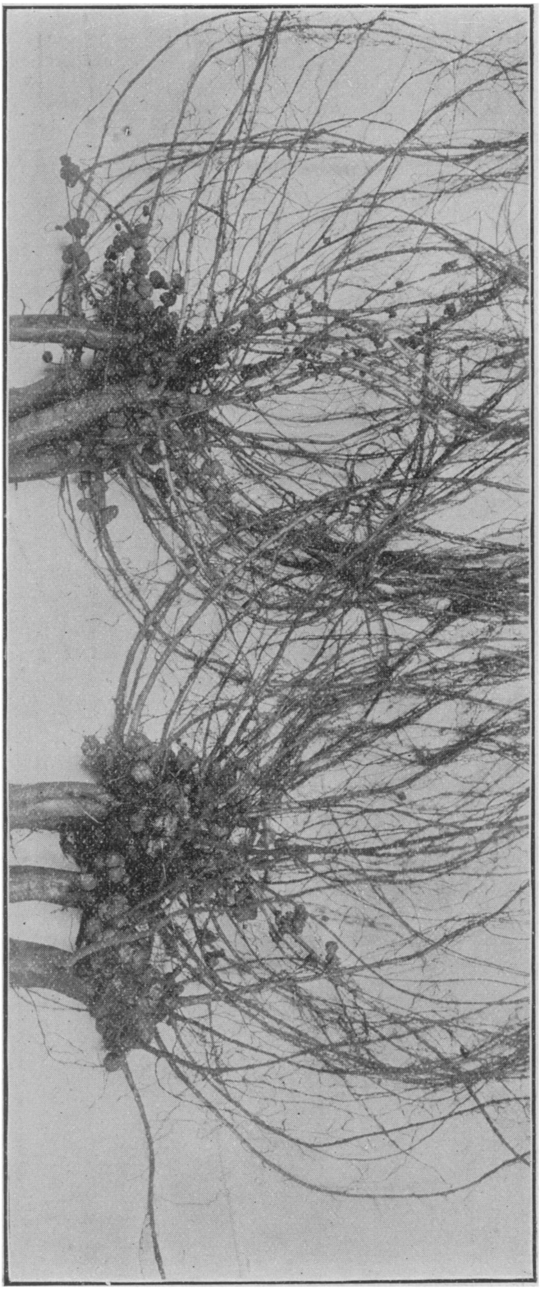


MEDIUM GREEN. YELLOW SOY.

I. SOY-BEANS NOT TREATED.

Transactions Kansas Academy of Science, Vol. XVI.

PLATE VII.

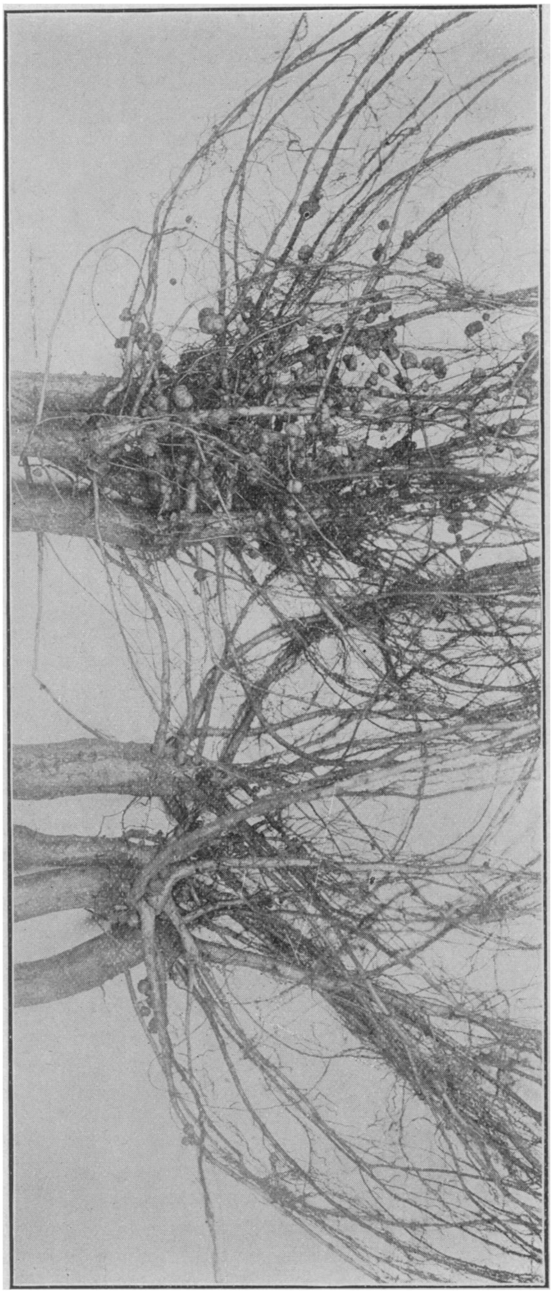


MEDIUM GREEN. YELLOW SOY.

II, SOY-BEANS INOCULATED WITH SOIL,

Transactions Kansas Academy of Science, Vol. XVI.

PLATE VIII.



MEDIUM GREEN. YELLOW SOY.

III. SOY-BEANS INOCULATED WITH EXTRACT.

Nitrogen Content.—On September 17 an average sample of six stalks each was taken from plats D and F of series II for analysis, with the purpose in view to ascertain whether there would be any difference in the content of nitrogen between the plants with tubercles and those without tubercles. The seed being the most constant in composition of any part of the plant, it was thought that the difference, if any, would be in the fodder, and so, after the samples were thoroughly dried, the beans were all shelled out and the fodder ground up fine. From this a sample was taken and pulverized for analysis. The per cent. of nitrogen is shown in the following table, together with the protein and water:

TABLE II.

Treatment.	Nitrogen, per cent.	Protein, per cent.	Water, per cent.
Inoculated with soil	1.439	8.996	7.89
Not treated	1.395	8.719	7.30
Difference	.044	.277	.59

The analysis does not show any great difference in favor of inoculating, there being an increase of only .04 of 1 per cent. of nitrogen and .27 of 1 per cent. of protein in favor of the beans with tubercles. This would be .8 pound nitrogen and 5.4 pounds protein increase for each ton. But it must not be concluded that this is the only difference. The roots with tubercles rich in nitrogen must possess greater fertilizing properties than the roots with no tubercles, the results of which would be shown in the succeeding crop or crops. Furthermore, had the tubercles been grown on poor soil instead of rich soil, doubtless there would have been a still greater difference in favor of inoculating. The remaining crop of the medium green was harvested October 2.

Data as to Yield.—When matured the beans were harvested and placed in gunny sacks to cure. The leaves had nearly all fallen off and a few of the pods were about ready to pop open, although many still had a green appearance. When both varieties had attained sufficient dryness the beans were thrashed out by hand and account taken of the weight of both grain and dry stalks. In case of the stalks the results cannot be considered entirely accurate, as many of the leaves had fallen off before the beans were fully ripe; and, furthermore, it was noticed that plats C and F, not treated, remained green longer than the inoculated plants, which tended to increase their fodder yield in comparison with the others. The results are shown in table III. From this table it will be seen that the yellow soys, plat B, inoculated with extract, yielded a little the best of both grain and fodder; but the difference is very slight. Of the medium green, plat F, not treated, yielded the most grain; and plat D, inoculated with soil, the most fodder. In all these cases the differences are not great, and, as the plats were very small, it would be impracticable to pass any judgment as to comparative yield. The benefits from inoculation lie largely in the increased fertility of the soil resulting from the decay of the nitrogenous roots, and would not be seen until after the growth of the succeeding crop.

EXPERIMENTS IN THE GREENHOUSE.

Repetition and Extension of Field Experiment.—Pots containing native soil were planted to beans and treated in the same manner as in the field experiment, and were attended with practically the same results. The test, in this case, was extended so as to include other varieties of the soy-bean, namely, the edamame, kiyusuke daidzu, yamagata cha-daidzu, early white, and the medium

TABLE III.

PLAT.	No. of stalks in plat.	Weight of grain, pounds.	Weight of dry stalks, pounds.	Weight in proportion to number of stalks.		Rate per acre.	
				Grain, pounds.	Stalks, pounds.	Grain, bushels.	Stalks, tons.
Yellow soy:							
A	85	2.875	4.562	3.246	5.152	23.27	1.10
B	73	2.625	4.312	3.451	5.669	24.74	1.21
C	100	3.437	5.312	3.299	5.099	23.65	1.09
Medium green:							
D	82	2.750	8.312	3.218	9.730	23.07	2.09
E	67	2.125	6.125	3.044	8.775	21.82	1.88
F	82	3.062	7.812	3.584	9.144	25.69	1.96

black. In all these cases, where the plants were inoculated with either soil or extract, numerous and well defined tubercles appeared on the roots. In a few instances, however, one or two tubercles were found on the plants not treated, but these were isolated cases and were undoubtedly due to infection resulting from the manipulation of tools and pots when the beans were planted.

HOW SOON DO THE TUBERCLES APPEAR?—To obtain information on this point, a small bed was planted in the greenhouse June 19 and inoculated with Massachusetts soil, from which plants were taken up nearly every day to ascertain when the tubercles began to appear. They were first visible to the naked eye on July 3, thirteen days after the beans were planted, or eight days after they appeared above the ground. From this it would be inferred that the bacteria begin their work very soon after the young roots are formed and increase their activity with the growth of the roots.

EFFECT OF STERILIZING THE SOIL.—Pots of both Kansas and Massachusetts soil were sterilized by heating them to 200° C. (392° F.) The results obtained, both in the field and in pots, as well as by previous experience, showed that as far as the soy-bean organism was concerned the Kansas soil was already sterile. In the case of the Massachusetts soil, however, these results showed that the bacteria were killed at the above temperature, and plants grown in this soil produced no tubercles except when inoculated. It might be well to state in this connection that the heating of the soil produced other effects than those of a bacteriological nature, and the plants grown in it did not possess a healthy and vigorous appearance.

PLANTS GROWN IN MASSACHUSETTS SOIL.—Since 21^{cc} of Massachusetts soil was capable of producing such good results, both in the field and in pots, it was thought that plants grown in this soil alone would give still more striking results in tubercle formation. One pot each of yellow soy and medium green were grown in Massachusetts soil. The plants did well and ranked among the best in the greenhouse, but on washing out the roots the tubercles were found to be only moderate in size but fairly well distributed over the roots. In fact they did not show up so well as plants which were inoculated with only a small portion of Massachusetts soil. This experiment is repeated and results given under the second series of experiments in the greenhouse.

INOCULATING AT TOP, MIDDLE, AND BOTTOM OF POT.—To test the rapidity with which the organisms spread in the soil, three pots each of yellow soy and medium green were inoculated at the top, middle, and bottom of the pots respectively with 21^{cc} of Massachusetts soil. The washing out of the roots revealed the fact that the plants inoculated at the top of the pot produced tubercles on the upper portion of the roots with only a few extending downward and none on the lower portion of the roots. The plants inoculated at the middle of the pot produced tubercles about midway between the upper and lower portion of the roots. And, lastly, the plants inoculated at the bottom of the pot showed the tubercles on the lower portion of the roots, with a few tending upward. This is a very interesting point, and indicates that, without mechanical mixing, the micro-organisms spread very slowly in the soil, and that in spite of the fact that the plants were frequently watered on upper surface of pot, which one might suppose would have carried the bacteria deeper into the pots. The number and position of the tubercles are shown in the accompanying drawings.

Fig. 1.
Soy-bean inoculated at top of pot.

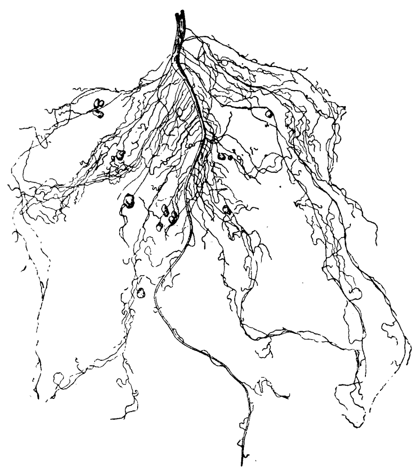


Fig. 2.
Soy-bean inoculated at middle of pot.

FURTHER EXPERIMENTS IN THE GREENHOUSE.

Plants Grown in Pure Massachusetts Soil.—Fearing that the results obtained in the previous experiment might be due to local conditions or disturbances, the subject was further tested by planting yellow soy-beans in seven pots of pure Massachusetts soil, and comparing with these seven pots of Kansas soil, all of which were inoculated with 21^{cc} of Massachusetts soil. The results obtained were similar to those of the previous experiment, only that no appreciable difference could be seen in the results of the two treatments. Why a soil so thoroughly infected with micro-organisms as was this Massachusetts soil should not cause greater development of tubercles is a question not readily answered, and one that will bear further investigation.

Inoculating with Different Amounts of Massachusetts Soil.—To test the effect of varying amounts of Massachusetts soil on the number and size of tubercles produced, ten pots of yellow soy-beans were grown in which the soil had been inoculated with 21^{cc} of Massachusetts soil for pot 1, 42^{cc} for pot 2, and so on, increasing 21^{cc} for each succeeding pot, until the tenth pot was reached, which received 210^{cc} of Massachusetts soil. No particular difference could be detected in the growth of the plants, and what was true of the upward growth was likewise found to be true of the roots and tubercles. The differences were slight, and these so irregular that it could not be said that one was any better than the others. These results, taken in connection with those obtained from pure Massachusetts soil, seem to indicate that the micro organisms are sufficiently numerous and active for ordinary inoculating in a comparatively small amount of the Massachusetts soil, and that an increase of this infectious soil does not perceptibly increase the number or size of the tubercles.

EFFECT OF LIGHT ON THE MICRO-ORGANISMS.—Two broad, shallow dishes, each with about 210^{cc} of the Massachusetts soil spread over their surfaces, were placed, one in diffused light and the other in sunlight, and enough to inoculate one pot was taken from each of these at the end of one, two, three, four, and six weeks, respectively. In the meantime the soil was kept stirred so as to expose all portions equally to the light. All pots contained tubercles; and, although the results slightly favor the pots whose inoculating material was exposed the least, the differences are very small. In fact, it seems that light could have but little effect on the micro-organisms when the soil is kept together in any quantity.

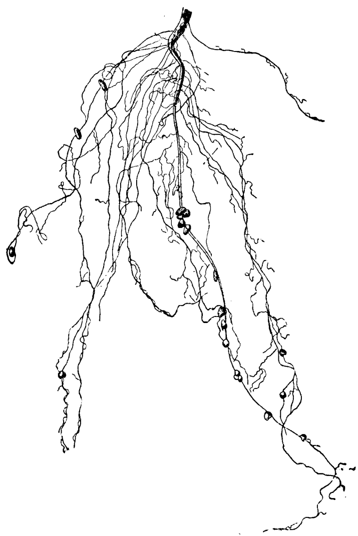


Fig. 3.
Soy-bean inoculated at bottom of pot.

INOCULATING AT DIFFERENT TEMPERATURES.—To see what degrees of temperature these micro-organisms could stand, soil was heated to ten different points, varying from 40° to 150° C. (104° to 302° F.) Tubercles were found in all the pots except 120° and 150° C. Unless the micro-organisms happened to possess less vitality in the former instance, the lack of tubercles could scarcely be attributed to the heat, as tubercles were found on the plants whose inoculating material was heated to 140° C. It was observed that the tubercles developed the best at the lower temperature and they seemed to decrease as the temperature increased, although this variation was not entirely regular. It would seem that some of the bacteria possessed more vitality than others and that the ones with less vitality were killed by the heat. A similar test was made by heating extract from 35° to 90° C. (95° to 174° F.); but as this was considerably lower than that to which the soil was heated, tubercles were formed in all the pots, as might be expected after the former discovery. But even here the same gradation existed as was noticeable in the case of the soil, the tubercles being more numerous at the lower temperatures. In both of these cases the results show that the micro-organisms can stand quite a high degree of heat.

INOCULATING WITH KANSAS SOIL.—Will soil which has once been inoculated serve to inoculate non-infected soils? First, five pots were filled with soil taken from the immediate vicinity of roots previously inoculated. Second, five pots were filled with soil which had been soaked and washed out from plants that had produced tubercles in the field. Since nearly a two-foot cube was taken up with each hill, the number of micro-organisms must have been less in this instance than in the first five pots. Tubercles were produced in all the pots; but the results, as might be expected, were somewhat more in favor of the first five. To test this matter still further, two pots were inoculated each with 21cc of the above classes of soil, with the result that in both cases tubercles were formed in the same relative proportion to the above. This shows that Kansas soil, being once inoculated, can be used to inoculate other soils.

INOCULATING WITH TUBERCULOUS ROOTS.—After remaining in loose soil about a month, some of the roots which had previously produced tubercles were taken to inoculate a pot of yellow soy-beans. The plants grew well and ranked among the best in the greenhouse. On washing out the roots, large and numerous tubercles were discovered, which were by far the best of any produced in the greenhouse during this experiment. Likewise, washed roots that had been air-dried in diffused light for about the same time were placed in another pot; tubercles were formed, but neither the growth of the plant nor the tubercles were equal to the above. In the former case the roots had more or less soil adhering to their surface, but in the latter there was practically none.

EFFECT OF INOCULATING OTHER LEGUMES WITH MASSACHUSETTS SOIL.—Four pots each of adzuki beans (*Phaseolus radiatus*), cow-peas, Canada field peas, alfalfa, and red clover were planted, half of these being inoculated with Massachusetts soil and the other half not treated. On the roots of the adzuki beans and the cow-peas no nodules were apparent in any of the pots; the alfalfa showed several; and on the clover and Canada field peas they were very numerous; but no difference could be detected on any of them that was due to the Massachusetts soil. Evidently these plants were attacked by a different kind of organism from that attacking the soy-bean.

ROOT TUBERCLES UNDER THE MICROSCOPE.

PREPARATION OF SLIDES.—This phase of the subject was taken up with the hope of observing the way micro-organisms behave within the tissues of the root. Tubercles were cut from the roots of plants seventy-two and ninety-nine days old, respectively, which had been grown in the greenhouse under rather unfavorable circumstances. These were placed in one per cent. chromic acid for eighteen hours, after which they were washed out and placed in fifteen per cent. alcohol for seventeen hours, then in thirty per cent. for nine hours, then fifty, sixty, eighty and ninety per cent., and absolute alcohol for six hours each, more or less, at convenience. They were then transferred to one-half alcohol and one-half turpentine for seven hours previous to placing them in pure turpentine. Following this treatment paraffine was added sufficient to make a saturated solution. This was placed on a radiator for twelve or fifteen hours to keep the paraffine melted and thus to more thoroughly saturate the tubercles, when they were removed to a water-bath and kept in paraffine at a temperature of fifty-eight degrees C. for two or three hours. The tubercles with the melted paraffine were then poured into a paper box, which was floated on the surface of water until the paraffine formed a scum on its upper surface, after which the whole was rapidly cooled by immersing it. From this solid paraffine pieces containing tubercles were cut out and mounted for the microtome.

When the sections were cut, they were placed on a glass slide previously covered with a thin coat of albumen solution to make them stick. This was then held over an alcohol lamp until the paraffine was all melted. After being allowed to cool, the paraffine was dissolved off with turpentine, and the specimen carried back through the various strengths of alcohol until it could be placed in water. It was then put into a solution of hæmatoxylon for twenty minutes to stain it and, after being brought up through alcohol to turpentine, was mounted in Canada balsam. The apparent infecting mycelium in the tubercle absorbed the stain more readily than the cell tissue, and could be seen with a Zeiss microscope fitted with 1-12 (2mm.) homogeneous objective and a No. 4 eyepiece. This gave a magnification of 850 diameters. Drawings representing cross-sections of the tubercles at this power were obtained with the aid of an improved Abbe camera. Specimens representing a portion of a cross-section of tubercles taken from plants seventy-two and ninety-nine days old are shown in figs. 4 and 5, respectively.

EXPLANATION OF MYCELIUM.—It should be noted that the mycelium that appears to run from cell to cell is a bacterial product and is therefore not a true mycelium. The apparent mycelium is what is known as a bacterioid condition; the bacteria become distributed throughout the cells and finally die. It is in this dead or decayed condition that the bacteria become available as plant food. The mycelium, or bacterioid condition, is the transition stage from the individual bacteria until their absorption by the plant. The change of the bacteria into the bacterioid condition is shown at *d*, fig. 5.

EXPLANATION OF MICROSCOPIC DRAWING.—Fig. 4 shows the cells, *a;* the nuclei, *b;* and the infecting mycelium (bacterioid condition), *c*. It will be noticed that the mycelium is formed through the cell-wall, appears to send off branches, and has a special liking for the cell nuclei. In the lower portion of

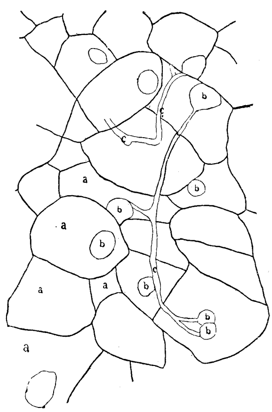


FIG. 4.
Cross-section of soy-bean root tubercle.

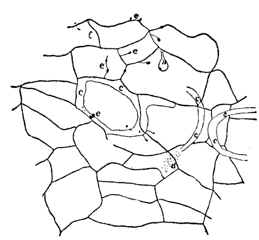


FIG. 5.
Cross-section of soy-bean root tubercle.

fig. 4 is shown the mycelium branching to two nuclei, one of which seems to lie below the other and many belong to a lower layer of cells. Fig. 5 shows a cross-section at a little later stage of development. As in the former case, it shows the mycelium, *c*, but in a little different form. In one instance the mycelium seems to envelop the inner wall of nearly all of one cell, and a portion extends through the cell wall into the adjoining cell. In addition to this, there is shown at *d* a cluster of small dots, which are probably individual bacteria. Also at *e* are found peculiar dark bodies, some of which are imbedded within the cell wall, while others are isolated or connected with threads, or hyphæ. The latter bodies may possibly be bacteria, but it seems to be more probable that they are something else. They may be due to some foreign substance that has the power of absorbing the stain to a greater degree than the surrounding tissues.

EXTENT OF SOY-BEAN MICRO ORGANISM IN THE UNITED STATES.

After the success of inoculating the beans with imported soil was assured, it was thought to be an interesting point to ascertain how far these particular micro-organisms had spread in this country. Accordingly inquiries were sent to all the experiment stations of the United States, and the following table constructed from the replies:

TABLE IV.

Micro-organisms indigenous to the soil.	Micro-organisms obtained through inoculation.	No tubercles found on the roots.	Have made no examination for root tubercles.	Too cold to successfully grow the soy-bean.	Have not grown the soy-bean.
Indiana	Conn. [Storrs],	California	Arizona	Minnesota	Kentucky
Louisiana	Kansas	Florida	Arkansas	Washington	Maine
Mass. [Hatch],		Iowa	Colorado		Montana
N. Carolina		Michigan	Conn. [State]		Nevada
Rhode Island,		South Dakota,	Georgia		Pennsylvania
Tennessee			Illinois		Utah
			Maryland		Virginia
			Missouri		Wyoming
			Mississippi		
			Nebraska		
			New Jersey		
			N. Y. [Cornell],		
			N. Y. [State]		
			Ohio		
			Texas		
			Vermont		
			West Virginia		
			Wisconsin		
6	2	5	18	2	8

CONCLUSION.

The above experiments were not planned with a view to obtain comparative results as to yields; and where yields have been given they are only incidental. The main object was to ascertain whether or not a leguminous plant could be made to produce tubercles by inoculating it with a soil impregnated with the right kind of micro-organisms. As the Kansas soil contained none of these organisms, the conditions were entirely under control, and results obtained which otherwise would have been impossible. The results show conclusively that inoculation is entirely possible; and this, taken in connection with the fact that it has been repeatedly proven that tubercles are valuable adjuncts to leguminous plants, both for yield and as a fertilizer, suggests the practicability of inoculating fields deficient in micro-organisms that would be beneficial to the particular leguminous crop to be grown.

When we realize that in the Eastern states many farmers are paying from six to ten dollars an acre for fertilizers, which in the aggregate amount to a tax of millions of dollars, and as we in the West are fast tending in the same direction, should it not behoove us to lay hold of one of nature's most effective means of maintaining and even increasing the fertility of the soil? Free nitrogen is around and about us in superabundance, it composing four-fifths of the air; but, without the aid of these bacteria working within the tubercles of the roots, plants have no power to make use of it. By growing leguminous crops in rotation, and inoculating the soil when the latter is deficient in the proper species of bacteria, and thus controlling the action of these microscopic plants, the farmer may find them to be among his best friends and strongest financial supporters.